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Mechanical and Thermal Properties of Sericin/PVA/Bentonite Scaffold: Comparison between Uncrosslinked and Crosslinked

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Summary: Clay aerogel is a highly porous material produced from smectite clays using freeze-drying techniques which produces a structure this is appropriated for 3D scaffolds. In this research, sericin and poly(vinyl alcohol) were blended with bentonite aerogels to develop the biomedical foam-liked materials for tissue engineering. Sericin/PVA/bentonite scaffolds were prepared by varying sericin contents in 1, 2, and 3 wt%. Glutaraldehyde was used as a chemical cross-linking agent in order to compare the properties of uncross-linked and cross-linked scaffolds. The morphology and crystal structure of sericin/PVA/bentonite scaffolds were identified by FE-SEM and XRD, respectively. The mechanical properties were characterized by compression tests and thermal properties were investigated by TG-DTA. The freeze-drying technique produced scaffolds with modulate modulus, interconnected pores, and pore sizes greater than 100 μm. Chemical crosslinking increased the mechanical properties but slightly decreased thermal stability of the scaffolds. Furthermore, crosslinking improved the structure stability of scaffolds in an aqueous solution.

Keywords: bentonite; clay aerogel; porous structure; scaffold; silk sericin

Introduction

Tissue engineering is an applied technique using a combination of engineering, cells and materials to create artificial framework for regeneration of new tissue. There are several requirements of scaffolds for tissue engineering such as biocompatibility, biodegradability, porous structure, etc. A highly porous structure with interconnected pores (pore diameter around 100 μm) is a considerable important parameter to create satisfactory scaffold.[1]

Aerogels are light weight and low density materials produced from gels in which the liquid is replaced with a gas and the solid network is not collapsed.[2] Clay aerogel is well known as the one type of aerogels which is generated from layered silicate clay from the smectite family through the simple freeze-drying technique. The highly porous structure between clay layers occurs from the alignment of ice crystals resulting in the "house of cards" liked structure with interconnected pores between each layer.[3,4] This material has drawn interest from researchers in the biotechnological field for use as drug control-release system or 3D scaffold because it meets several criteria of these applications. However, neat clay aerogels form into fragile and brittle material and are therefore difficult to handle and collapse easily under low stress levels. In order to overcome this limitation, blending with other materials like reactive or crosslinkable polymers e.g. poly(vinyl alcohol) or PVA has been used to improving the strength of these materials.[3–6]

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Sericin is the "glue like" protein constituted about 20–30% in silk cocoons (*Bombyx mori*). Sericin is extracted from silk fiber (fibroin) by degumming processes and generally discarded as waste water causing environmental pollution.[7] Sericin is renowned as a natural hydrophilic protein with many outstanding properties in biomedical applications. Mandel *et al.* (2009), Aramwit *et al.* (2010) and Takeuchi *et al.* (2005) used sericin to promote cell growth and cell regeneration in tissue engineering applications due to its biocompatibility, biodegradability and hydrophillicity. The hydrophillicity of sericin can facilitate cell attachment on the surface of the scaffold because it can promote the penetration of an aqueous cell suspension.[8–11]

In this study, bentonite (BTN) clay was combined with PVA and sericin to develop novel biodegradable aerogel materials (prepared by freeze-drying technique) for application as scaffolds. Furthermore, glutaraldehyde, a chemical crosslinking reagent in most biopolymers, was employed for the crosslinking of sericin and PVA to improve the mechanical properties as well as the insolubility of the aerogel scaffolds which was important for cell culture and cell cytotoxicity tests. This study concentrated on the influence of sericin content with and without chemical crosslinking agent on the mechanical and thermal properties of the aerogel scaffolds.

Experimental Part

Materials

Silk cocoons (*B. mori*) were obtained from native Thai silk agriculture. Bentonite with cationic exchange capacity (CEC) of 49.74 meq/100 g clay, was kindly supplied from Thai Nippon Chemical Industry Co., Ltd. Poly(vinyl alcohol) was purchased from KURARAY POVAL (hydrolyzed value at 87–89 mol%) with average molecular weight of 9000–10000 g/mole (characterized by GPC) and viscosity is 40–48 mPa.s (4% aqueous solution at 20 °C). Glutaraldehyde ($C_5H_8O_2$, CAS No.111-30-8) used as crosslinking agent was purchased from Sigma Aldrich Corp., USA.

Sericin Extraction

Silk cocoons were cut into small pieces about 5×5 mm^2 and mixed with purified water. Then, silk cocoon pieces were autoclaved under pressure of 0.8–0.9 atm at 120 °C for 60 min. The fibroin was filtered out to obtain the sericin solution. This method was repeated twice. Sericin solution was frozen in the glass vials at −40 °C for 12 hr. After freezing, the shells were put into a freeze-dryer maintained at −110 °C for 48 hr. Freeze-dried silk sericin was ground into powder.

Sericin/PVA/Bentonite Scaffolds Preparation

Sericin powder with various contents (1–3 wt%) and PVA (5wt%) were dissolved in purified water and heated at 90 °C until the mixture was completely dissolved. The bentonite was added followed by vigorous stirring for 2 hr. After cooling to the ambient temperature, the sericin/PVA/bentonite mixture was immediately frozen in cylindrical glass vials at −40 °C for 12 hr and attached to a freeze-dryer maintained at −110 °C for 72 hr.

Crosslinked Sericin/PVA/Bentonite Scaffold Preparation

Uncrosslinked sericin/PVA/bentonite mixture was prepared as defined above. Then, glutaraldehyde (GT) at concentration of 7 μl/ml of the mixture was added into the mixture under constant stirring for 1 hr. The mixture was cooled down to ambient temperature and subjected to the freeze-dried procedure as mentioned above. After 72 hr in the freeze-dryer, the samples were removed and post cured at 120 °C to ensure the maximum curing and the residual glutaraldehyde was removed.

The uncrosslinked samples were coded as CxP5Sy where x and y corresponded to clay and sericin contents (wt%), respectively. For example, C8P5S1 would be composed of clay 8 wt%, PVA 5 wt% and sericin 1 wt%.

The glutaraldehyde-crosslinked samples were denoted by 7 μl/ml of GT.

Characterization

The density of the sericin/PVA/bentonite aerogel scaffold was calculated by mass and dimension measurement using Sartorius BS 224 S analytical balance and digital vernier caliper according to an equation:

$$\rho = \frac{M}{V}$$

where ρ is mass density (g/cm^3), M is mass of sample (g) and V is volume of sample (cm^3).

The morphology of the scaffolds was examined using a Hitachi S-4800 field emission scanning electron microscope (FE-SEM). The samples were coated with platinum under vacuum. FE-SEM micrographs were taken with the magnification range between 45-20,000 using an accelerator voltage of 2.0 kV.

The structure of the scaffold was characterized using a Bruker AXS Model D8 Discover X-ray diffractometer. The X-ray beam was Cu (k = 0.15406 nm), and the radiation operated at a tube voltage of 40 kV and a tube current of 30 mA. The samples were scanned in a step mode at a scan rate of 2°/min from $2\theta = 5°–30°$. The interlayer spacing (d_{001}-spacing) was calculated via the Bragg equation.

Thermal stability of the scaffold was investigated by Perkin-Elmer Pyris Diamond thermogravimetric analysis. The weight of the sample was in the range of 5–7 mg and heated at the heating rate of 10 °C/min from 30–900 °C in a nitrogen atmosphere with nitrogen flow rate of 30 ml/min.

The initial modulus of the scaffolds was investigated by a LLOYD LRX universal testing machine in compression mode with a 500 N load cell at a constant crosshead speed of 1 mm/min. The samples were prepared in the cylindrical shape with a ~20 mm diameter and height. Five samples of each composition were tested for reproducibility. The initial compressive modulus was calculated from the slope of the linear portion of the stress-strain curve.

Results and Discussion

Morphology of Sericin/PVA/Bentonite Scaffolds and Dispersion of Clay in Scaffolds

Sericin/PVA/bentonite scaffolds, both uncrosslinked and GT crosslinked, showed the lamella morphology with the clay layers covered by sericin and PVA and a pore size greater than 100 μm. The FE-SEM micrographs of sericin/PVA/bentonite scaffolds are shown in Figure 1A and 1B. At small sericin contents (1–2 wt%), the lamella morphology was clearly observed with small amounts of polymer webs connecting between each layer. With an increased sericin content (3 wt%), the clay layers were highly covered by polymer and layers appeared to be linked by webs of polymer; as a result, the distance between layers and pore size was decreased.[12] These results suggested that the morphology of the scaffold was highly sensitive to the polymer (sericin and PVA) contents. The network of GT crosslinked sericin and PVA on the layers of clay is shown in Figure 1C. The crosslinking reaction by GT can occur between sericin or PVA itself, sericin and PVA, sericin and silanol groups of bentonite and also between PVA and silanol groups of bentonite.

The XRD patterns of Na-bentonite and sericin/PVA/clay scaffold are shown in Figure 2. The bentonite showed the diffraction peak at 2θ around 7°corresponding to a d_{001} spacing of 1.25 nm.[5,13] Moreover, PVA and sericin exhibited diffraction peaks at around 19.6° and 20°, respectively.[3,8,13] The sericin/PVA/clay scaffold showed the reduced intensity of the characteristic peaks of bentonite due to the dilution effect. According to Bandi *et al.*, 2006 and Pojanavaraphan *et al.*, 2010, the shift of diffraction peaks to the lower angle around $2\theta = 4°$ that is observed suggests a change in the d_{001} spacing. Therefore, the intercalation of polymers inside the clay platelets has occurred. When bentonite was swollen in solvent (water) and mixed with polymers (sericin and PVA), the polymer chains would be intercalated and substitute the

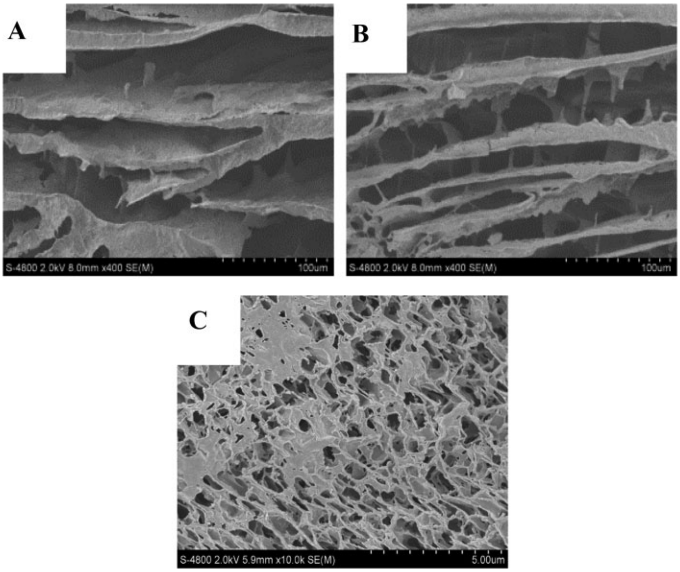

Figure 1.
FE-SEM micrographs of sericin/PVA/bentonite scaffolds: A) C6P5S1, B) C6P5S3 and C) C6P5S1 7GT the network of sericin and PVA on clay layers.

solvent in the bentonite galleries resulting in the intercalation structure by a solution process.

Thermal Stability of Sericin/PVA/ Bentonite Scaffold

The effects of sericin and chemical crosslinking on the thermal stability were studied by thermogravimetric analysis. Figure 3 and Table 1 show the TGA results of sericin/PVA/bentonite scaffolds with various sericin concentrations, with and without GT crosslinks.

The scaffolds showed two main steps of weight loss. First, the gradual mass loss observed up to 100 °C corresponded to the evaporation of water in the bulk samples (~1–2% weight loss) followed by the second

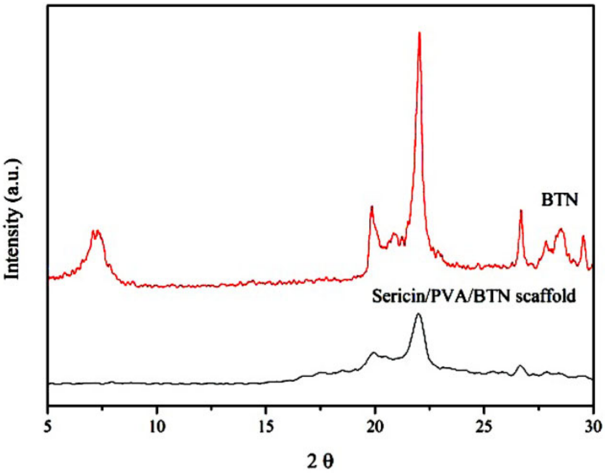

Figure 2.
XRD patterns of pure Na-bentonite and C6P5S1 silk sericin/PVA/bentonite aerogel scaffold.

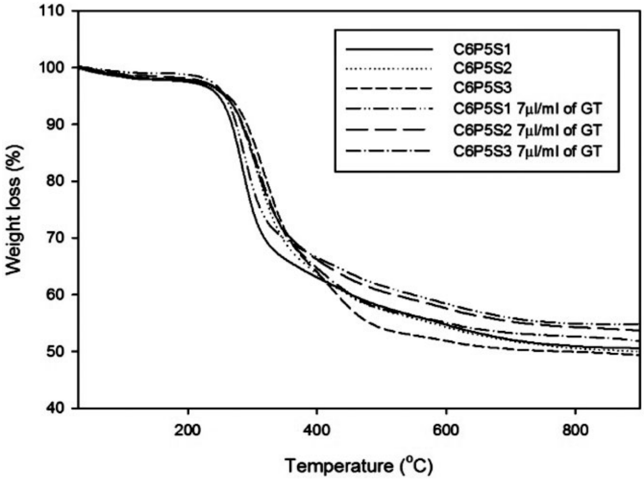

Figure 3.
Thermogravimatic curves of sericin/PVA/bentonite scaffolds by varying sericin contents, with and without GT crosslinking.

step around 270 °C which is associated with the decomposition of silk sericin (~260 °C) and PVA (~280 °C). As given in Table 1, the sample without sericin (PVA/bentonite aerogel, C6P5S0) shows lower the onset temperature of decomposition than sericin/PVA/bentonite samples. The onset and maximum decomposition temperatures of the scaffolds significantly increased when the sericin concentration increased. This occurrence is attributed to the increasing number of hydrogen bonds between sericin, PVA molecules and silanol groups of bentonite which are important factors for the promotion of the thermal stability of the scaffold.[14] It is worth nothing that as sericin content increases, the more decomposable organic content exists thus resulting in smaller residue content.

Table 1.
Thermal behavior of sericin/PVA/bentonite scaffolds.

Samples	T_d onset (°C)	Max. T_d (°C)	Char residual (%)
C6P5S0	205.2	–	53
C6P5S1	255.9	285.7	51
C6P5S2	266.6	305.8	50
C6P5S3	273.1	317.6	49
C6P5S1 7GT	257.4	289	54
C6P5S2 7GT	262.9	301.8	53
C6P5S3 7GT	264.7	306.4	51

The thermal stability of the GT cross-linked sericin/PVA/bentonite scaffolds shows less water absorption content suggesting less polarity due to functional sites used during the crosslinking reaction. In addition, the decomposition was shifted to higher temperature and a higher residue content than those of the uncrosslinked samples although cross-linking by glutaraldehyde at higher sericin content (2–3 wt%) brought about a slight decrease in the onset and peak decomposition temperatures. This better thermal stability of the cross-linked scaffolds supports the success of crosslinking reactions taken placed in PVA and sericin by glutaraldehyde.

Mechanical Properties of Sericin/PVA/Bentonite Scaffolds

The sericin/PVA/bentonite scaffold presented low density materials owing to the high porosity of the scaffold which is the main characteristic of the clay aerogel. The density of the scaffolds was around 0.11–0.13 g/cm^3 depended on the sericin content and chemical cross-linking; hence the sericin/PVA/bentonite scaffolds had modulated moduli. The compressive stress-strain curves of uncrosslinked and GT crosslinked sericin/PVA/bentonite scaffolds are shown in Figure 4. The sericin/PVA/bentonite

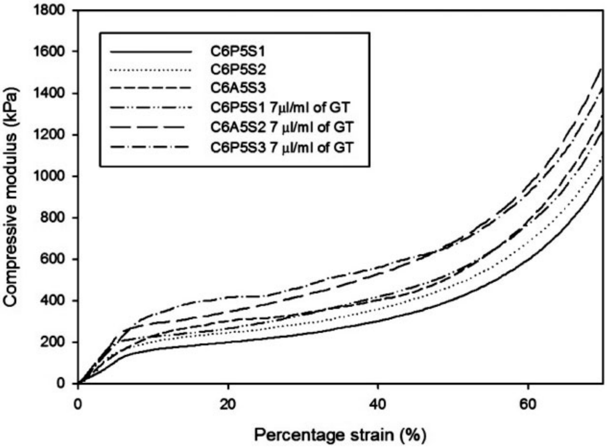

Figure 4.
Stress-strain curves of sericin/PVA/bentonite scaffold, comparison between sericin content with and without GT crosslinking.

scaffolds have the stress-strain curves that follow the pattern of a classical rigid foam-liked behavior. At low strain (initial loading), scaffolds showed linear-elastic deformation. Increasing the compression load, the horizontal plateau was followed by the densification region where the voids were wholly distorted.[4–6] The scaffolds then exhibited non-elastic behavior; the shape of the aerogels were not recovered after the compression testing. Table 2 and Figure 5 summarize the mechanical properties of PVA/bentonite and sericin/PVA/bentonite scaffolds. As give in Table 2, with the presence of sericin, the moduli of the scaffolds are significantly improved. Moreover, the sericin contents and chemical crosslinking strongly affected the mechanical properties. Increasing content of silk sericin vastly increased the mechanical properties of the scaffolds because the high content of sericin increased the formation of hydrogen bonds between the sericin chains and the sericin and PVA chains. Additionally, the interpenetrating co-continuous networks were enlarged at high sericin contents.[13]

In the same way, the crosslinking reaction by GT improved the mechanical properties especially in the case of high loading of sericin. When comparing the uncrosslinked and GT crosslinked sericin/PVA/bentonite scaffolds, GT cross-linked scaffolds showed approximately 15–300% improvements in initial modulus depending on sericin loading. According to Pojanavaraphan *et al.*, 2010, the crosslinked network in the crosslinked scaffold system could dissipate energy more efficiently under the

Table 2.
Mechanical properties of PVA/bentonite and sericin/PVA/bentonite scaffolds.

Samples	Density (g/cm^3)	Initial modulus (kPa)	Young's modulus (kPa)
C6P5S0	–	976 ± 297	1551 ± 380
C6P5S1	0.112 ± 0.002	1869 ± 320	5970 ± 670
C6P5S2	0.129 ± 0.007	2020 ± 584	6110 ± 140
C6P5S3	0.139 ± 0.004	2753 ± 73	7380 ± 650
C6P5S0 7GT	–	1758 ± 533	2291 ± 531
C6P5S1 7GT	0.133 ± 0.007	2943 ± 308	6329 ± 779
C6P5S2 7GT	0.133 ± 0.015	3673 ± 102	7705 ± 875
C6P5S3 7GT	0.134 ± 0.004	4671 ± 710	7903 ± 430

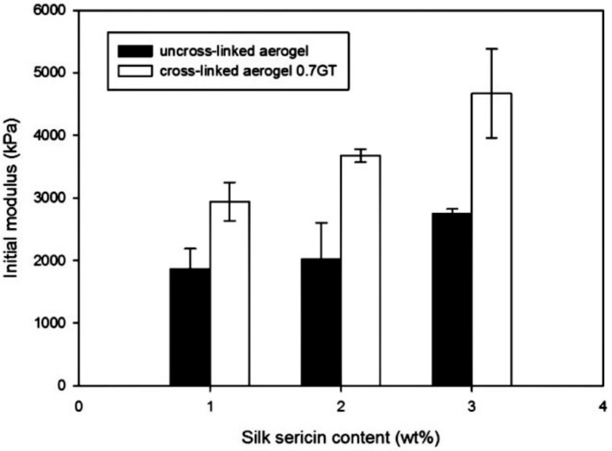

Figure 5.
Initial modulus of uncrosslinked and crosslinked sericin/PVA/bentonite scaffolds plotted as a function of sericin content.

applied load in comparison to the uncrosslinked scaffolds.

Conclusion

The sericin/PVA/bentonite aerogels were prepared via the simple freeze drying technique. The lamella structure (house of cards structure) with interconnected pore of sericin/PVA/clay aerogels were appropriated for medical applications. The addition of sericin into the scaffolds clearly promoted both thermal and mechanical properties as compared to the scaffold without sericin. By increasing sericin contents, the mechanical and thermal properties could be further improved. The chemical crosslinking by glutaraldehyde powerfully further enhanced the mechanical properties of the scaffolds but not significantly affected on thermal stability. The biological test is required in order to confirm the opportunity to use these aerogels as scaffolds for tissue engineering application.

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[1] S. Yang, K. F. Leong, Z. Du, C. Chua, *Tissue. Eng.* **2001**, *7*, 679–689.
[2] M. A. Aegerter, N. Leventis, M. M. Koebel, in: "*Aerogels Handbook*", 1st ed., Springer, New York **2011**, 3–17
[3] S. R. Hostler, A. R. Abramson, M. D. Gawryla, S. A. Bandi, D. A. Schiraldi, *Inter. J. Heat and Mass Transf.* **2009**, *52*, 665–669.
[4] T. Pojanavaraphan, R. Magaraphan, B. S. Chiou, D. A. Schiraldi, *Biomacromolecules* **2010**, *11*, 2640–2646.
[5] T. Pojanavaraphan, D. A. Schiraldi, R. Magaraphan, *Appl. Clay. Sci.* **2010**, *50*, 271–279.
[6] T. Pojanavaraphan, R. Magaraphan, *Eur. Polym. J.* **2008**, *44*, 1968–1977.
[7] S. C. Kundu, B. C. Dash, R. Dash, D. L. Kaplan, *Prog. Polym. Sci.* **2008**, *33*, 998–1012.
[8] Y. Zhang, *Biotechnol. Adv.* **2002**, *20*, 91–100.
[9] B. B. Mandel, A. S. Priya, S. C. Kundu, *Acta Biomaterials* **2009**, *5*, 3007–3020.
[10] P. Aramwit, T. Siritientong, S. Kanokpanont, T. Srichana, *Int. J. Biol. Macromol.* **2010**, *47*, 668–675.
[11] A. Takeuchi, C. Ohtsuki, M. Kamitakahara, S. Ogata, A. Toshiki, M. Miyazaki, Y. Furutani, H. Kinoshita, *Key. Emg. Mat.* **2005**, *284-286*, 329–332.
[12] K. Finlay, M. D. Gawryla, D. A. Schiraldi, *Ind. Eng. Chem. Res.* **2008**, *47*, 615–619.
[13] S. M. Alhassan, S. Qutubuddin, D. A. Schiraldi, *Langmuir.* **2010**, *26*, 12198–12202.
[14] H. Teramoto, A. Kakaza, K. Yamauchi, T. Asakura, *Macromolecules* **2007**, *40*, 1562–2569.
[15] W. Tao, M. Li, R. Xie, *Macromol. Mater. Eng.* **2005**, *290*, 188–194.